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Anticonvulsant and neuroprotective effect of (*S*)-3,4-dicarboxyphenylglycine against seizures induced in immature rats by homocysteic acid

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Abstract

The present study has examined the anticonvulsant and neuroprotective effect of (*S*)-3,4-dicarboxyphenylglycine ((*S*)-3,4-DCPG), a highly selective agonist for subtype 8 of group III metabotropic glutamate receptors (mGluRs), against seizures induced in immature 12-day-old rats by bilateral icv infusion of DL-homocysteic acid (DL-HCA, 600 nmol/side). For biochemical analyses, rat pups were sacrificed during generalized clonic-tonic seizures, ~45–50 min after infusion. Comparable time intervals were used for sacrificing the animals which had received (*S*)-3,4-DCPG (0.25 nmol/each side, 15–20 min prior to infusion of DL-HCA or saline). This agonist provided a pronounced anticonvulsant effect, generalized clonic-tonic seizures were completely suppressed and cortical energy metabolite changes which normally accompany these seizures were either normalized (decrease of glucose and glycogen) or markedly reduced (an accumulation of lactate). Anticonvulsant effect of (*S*)-3,4-DCPG was also evident from the EEG recordings, nevertheless, it was not complete. In spite of the absence of obvious motor phenomena, sporadic ictal activity could be seen in some animals. Isolated spikes could also be observed in some animals after administration of (*S*)-3,4-DCPG alone. The neuroprotective effect of (*S*)-3,4-DCPG was evaluated after 24 h and 6 days of survival following DL-HCA-induced seizures. Massive neuronal degeneration was observed in a number of brain regions following infusion of DL-HCA alone (seizure group), whereas pretreatment with (*S*)-3,4-DCPG provided substantial neuroprotection. The present findings suggest that receptor subtype 8 of group III mGluRs may be considered a promising target for drug therapy in childhood epilepsies in the future.

Keywords: Immature rats; DL-homocysteic acid-induced seizures; EEG recordings; Energy metabolites; Neuronal degeneration; mGluR8 agonist (*S*)-3,4-DCPG; Protection

1. Introduction

Metabotropic glutamate receptors (mGluRs) are a heterogeneous family of G-protein-coupled receptors that are widely distributed throughout the CNS (Conn and Pin, 1997; Schoepp et al., 1999) and the function of which is not to mediate but rather to modulate brain excitability via presynaptic, postsynaptic and glial mechanisms (Bruno et al., 2001; Schoepp, 2001). To date, eight mGluR subtypes (mGluR1–mGluR8) together with slice variants have been cloned and they are divided into three groups, defined on the basis of their amino acid sequence homology, signal transduction mechanisms, and agonist selectivity (Nakanishi, 1992; Pellicciari and Costantino, 1999).

Metabotropic glutamate receptors have in recent years been considered as potential targets for neuroprotective and/or anticonvulsant drugs. It has been postulated that ligands interacting with mGluRs might be devoid of undesirable side effects observed after the administration of antagonists for ionotropic glutamate receptors since they do not hamper the efficacy of

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fast excitatory synaptic transmission (Bruno et al., 2001). Literary data indicate that the pharmacological blockade of group I mGluRs or pharmacological activation of group II and/or group III mGluRs produces neuroprotection in a variety of in vitro and in vivo conditions (Bruno et al., 2001; Schoepp, 2001; Moldrich et al., 2003).

As to the group III mGluRs, anticonvulsant effects have been observed in various models of seizures after application of agonists for group III mGluRs (Tizzano et al., 1995; Ghauri et al., 1996; Abdul-Ghani et al., 1997; Thomsen and Dalby, 1998; Chapman et al., 1999, 2001; Gasparini et al., 1999; Folbergrová et al., 2001, 2003; Moldrich et al., 2001). We have demonstrated that administration of group III mGluR agonist (*R*,*S*)-4-phosphonophenylglycine ((*R*,*S*)-PPG) had a pronounced anticonvulsant effect against seizures induced in immature rats by homocysteic acid (Folbergrová et al., 2003). In addition, massive neuronal degeneration associated with this model of seizures (Langmeier et al., 2003; Folbergrová et al., 2005) was markedly attenuated after the pretreatment with (*R*,*S*)-PPG (Langmeier et al., 2006). However, the precise identity of group III mGluR subtype(s) (mGlu4, mGlu8 or both?) responsible for the anticonvulsant and neuroprotective effect of (*R*,*S*)-PPG is not clear. It seems thus important to examine further the role of individual mGluR subtypes, employing more selective agonists.

(*S*)-3,4-Dicarboxyphenylglycine ((*S*)-3,4-DCPG) has been identified as a potent and highly selective agonist for mGlu8 receptor subtype of group III, having little activity on group I or II mGlu receptors and displaying at least 100-fold selectivity for mGlu8 over the other group III mGlu receptor subtypes (Thomas et al., 2001). The aim of the present study was to examine the potential anticonvulsant and neuroprotective effect of (*S*)-3,4-DCPG against seizures induced in immature rats by intracerebroventricular (icv) infusion of DL-homocysteic acid (DL-HCA). Anticonvulsant activity of this agonist has been reported against sound-induced seizures in DBA/2 mice (Moldrich et al., 2001).

The anticonvulsant effect of (*S*)-3,4-DCPG was evaluated in terms of the suppression of behavioral symptoms of seizures, the recordings of bioelectrical activity, and the protection of cortical energy metabolite changes which normally accompany these seizures (Folbergrová et al., 2000, 2003). The neuroprotective effect of (*S*)-3,4-DCPG was evaluated on the basis of amelioration of neuronal degeneration associated with this model of seizures (Folbergrová et al., 2005, 2006).

Some of these findings have been presented in abstract form elsewhere (Folbergrová et al., 2004).

2. Materials and methods

2.1. Animals

Immature 12-day-old male Wistar albino rats were used for these experiments. The animals were anesthetized by ether and fixed in a stereotaxic apparatus, modified for rat pups and further operations were performed as described previously (Folbergrová et al., 2000). Briefly, bilateral stainless steel guide cannulae (26-gauge, 5 mm in length) were stereotaxically implanted 1 mm above the lateral ventricles (AP: 0.2–0.3 mm caudal from the bregma; L: ±1.6 mm; V: 3.3 mm from the skull surface). The cannulae were secured with fast-curing dental acrylic and the pups were allowed a minimum of 90 min to recover from surgery. Seizures were induced by bilateral intracerebroventricular (icv) infusion of DL-HCA (600 nmol/side) using stainless steel internal cannulae (33-gauge, 6 mm in length), each connected by a polyethylene tube to a 10 μl Hamilton syringe. Infusions of DL-HCA, (*S*)-3,4-DCPG or saline were made in a volume of 0.5 μl at a rate of 0.17 μl/min using an SP200i infusion pump (WPI, USA). The correct placement of both cannulae was histologically checked routinely in all brains, with the exception of those used for biochemical analyses.

For electrophysiological recordings, in addition to the cannulae, silver electrodes were implanted epidurally over the frontal cortex and stereotaxically introduced into the dorsal hippocampus of both hemispheres. The hippocampal electrodes were isolated up to their tips. A reference electrode was inserted into the nasal bone, the grounding electrode into the occipital bone over the cerebellum. The whole assembly was fixed to the skull by means of a fast curing dental acrylic. Animals were allowed to recover for at least 90 min after the surgery. Rat pups were placed individually into plastic boxes and connected to a paperless EEG apparatus (Kaminskij-Biomedical Research Systems, Prague, Czech Republic). The apparatus enabled to register the bioelectrical activity simultaneously from eight animals, always comprising pups from each experimental group, i.e. DL-HCA alone, DL-HCA plus (*S*)-3,4-DCPG, (*S*)-3,4-DCPG alone and control rats with saline. EEG signal, after amplification and filtering (0.15–75 Hz), was digitalized at a rate of 200 Hz and saved on the harddisc. Baseline activity was recorded for 5 min before the icv infusions. Recordings continued for at least 2 h. Analysis was performed offline with a software provided by the producer of the system.

The protocol of the experiments was approved by the Animal Care and Use Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic to be in agreement with the Animal Protection Law of the Czech Republic, which is fully compatible with the guidelines of the European Community Council directives 86/609/EEC. The Institute possesses The Statement of Compliance with Standards of Humane Care and Use of Laboratory Animals #A5228-01 from NIH. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Materials

DL-HCA (from Aldrich) was dissolved in saline and after adjusting the pH to ~7.0, only freshly prepared solutions were used. For evaluating anticonvulsant activity, potent and highly selective group III mGluR8 agonist (*S*)-3,4-DCPG (from TOCRIS Cookson, Bristol, UK) was used. The drug was dissolved in redestilled water containing a calculated amount of 5N NaOH to attain solubilization and approximate pH 7.0. (*S*)-3,4-DCPG was given by bilateral icv infusions (0.5 μl/each side; 0.25 nmol/each side) at 15–20 min intervals prior to icv infusion of DL-HCA or saline. Control animals received corresponding volumes of saline. Selection of (*S*)-3,4-DCPG dose was based on the data previously reported for adult animals (Moldrich et al., 2001) and our own data obtained during testing anticonvulsant efficacy of (*S*)-3,4-DCPG in immature rats (Table 1 and unpublished results).

2.3. Experimental groups

The animals were randomly divided into four experimental groups (10 animals in each group for the behavioral studies, 5 animals for the biochemical studies, 5–7 animals for electrophysiological recordings and 4–8 animals for histological analyses in each group): a) control group with saline; b) animals given (*S*)-3,4-DCPG alone; c) seizure group (with DL-HCA alone); and d) animals given (*S*)-3,4-DCPG plus DL-HCA. It should be noted that animals in groups b, c and d were given two injections (substitution of vehicle for DL-HCA in group b) and for (*S*)-3,4-DCPG in group c)). In the behavioral studies, the rat pups were observed for 2–3 h after infusion of DL-HCA and then were sacrificed by an overdose of ether. Bioelectrical activity was recorded for 2 h and then the rat pups were sacrificed by an overdose of ether. The pups for biochemical studies were killed during the period of generalized clonic-tonic seizures, approximately 45–50 min after icv infusion of DL-HCA (group c).